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Evolution of Short-lived and Long-lived Races of *Drosophila* in the Environs of Laboratory

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ABSTRACT

Aging is no more an intractable process and it can be better understood by life span studies and interventions like dietary restriction in model organisms. The aim of this study was to determine the stability of lifespan in the laboratory evolved cytoraces of nasuta-albomicans complex of Drosophila. These cytoraces were subjected for the following lifespan assessments: a) three independent replicate assessments with standard food media; b) validation of short-lived and long-lived cytoraces by crossing experiments; and c) response of lifespan to dietary restriction with diluted yeast in the food media. The findings were: 1) establishment of cytoraces 3 and 15 as short-lived and cytoraces 2, 9, 11 and 16 as long-lived by three replicate lifespan assessments; 2) nonsignificant differences in lifespan of F1 offspring of short-lived as well as two long-lived crosses from their parents; 3) extension of lifespan in short-lived races, but not in long-lived races in response to dietary restriction. Thus, the evolution of new short-lived and long-lived cytoraces and their differential response to dietary restrictions could be due to rapid genomic changes that had taken place during introgression via hybridization.

Keywords : Cytoraces, Dietary restriction, Hybridization, Introgression, Nasuta-albomicans complex.

Lifespan and its influencing factors like humidity (Pearl and Parker, 1922), light density (Northrop, 1925), population density (Pearl *et al.*, 1927), nutrition (David and Fouillet, 1971) ultraviolet and ionizing radiation

(Gartner, 1973), temperature (Parsons, 1977) and larval crowding (Luckinbill and Clare, 1985) have been widely studied in Drosophila melanogaster. Differences in lifespan have been reported for both inter- and intra-specific variations among the D. melanogaster, obscura and virilis species groups (Durbin and Yoon, 1986, 1987). Since 1990's genetic screening efforts with invertebrates have unraveled multiple genetic pathways that suggest longevity is promoted through the manipulation of diet metabolism and the resistance to oxidative stress to those based on the pro-senescence role of genes important for fitness early in life (Charlesworth, 1993; Chippindale *et al.*, 1993; Chapman and Partridge, 1996; Sohal and Weindruch, 1996; Parkes *et al.*, 1998; Rogina *et al.*, 2000; Tatar *et al.*, 2003; Partridge and Gems, 2006; Paaby and Schmidt, 2009). Among all these interventions, dietary restriction is a potent regimen in extending lifespan in Drosophila melanogaster and it can be achieved by diluting yeast, the major source of protein, vitamins, lipids and cholesterol in adult nutrient media (Chippindale *et al.*, 1993; Chapman and Partridge, 1996).

To study aging and its interventions through dietary restriction, nasuta-albomicans complex (NAC) of Drosophila offers a unique opportunity, since they are the hybrid recombination products. The evolution of this complex of Drosophila took place in the environs of laboratory through interracial hybridization between D.n.nasuta and D.n.albomicans which are morphologically identical, cross fertile karyotypically dissimilar (D.n.nasuta 2n=8: ?= 2^n2^n X^n Y^n 3^n 3^n 4^n 4^n ; ?=2^n 2^n X^n X^n 3^n 3^n 4^n 4^n; D.n.albomicans 2n=6: ?= 2^a2^a $X3^a$ $Y3^a$ 4^a 4^a ; ?= 2^a 2^a $X3^a$ $X3^a$ 4^a 4^a, where 'n' and 'a' represents D.n.nasuta and D.n.albomicans chromosomes, respectively) immigrans species of nasuta subgroup of Drosophila. The hybrid products showed karyotypic mosaicism, but after F_{20} – F_{50} generations it was declined and karyotypically stabilized four hybrid cytoraces 1, 2, 3 and 4 were evolved (Ramachandra and Ranganath, 1986, 1990). Further, interracial hybridization was made among the newly evolved four cytoraces, *D.n.nasuta* and *D.n.albomicans*, which resulted in the formation of twelve new cytoraces 5 to 16; all these members were then together termed as *nasuta-albomicans* complex (*NAC*) of *Drosophila*

(Ramachandra and Ranganath, 1996). Based on the karyotypic homology, sixteen cytoraces were grouped under six types (Tanuja *et al.*, 2003) namely, Type 1 (M 2n = 7 - 2^n 2^a X3^a Y^n 3^n 4^n 4^n ; F : 2n = 6 - 2^n 2^a X3^a X3^a 4^n 4^n), Type 2 (M : 2n = 6 - 2^n 2^a X3^a Y3^a 4^a 4^a ; F = 2n = 6 - 2^n 2^a X3^a X3^a 4^a 4^a) , Type 3 (M = 2n = 8 - 2^n 2^a X^n Y^n 3^n 3^n 4^a 4^a; F = 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^a4^a), Type 4 (M = 2n = 7 - 2^n 2^a Y3^a X^n 3^n 4^a 4^a ; F = 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^a 4^a), Type 5 (M=2n = 7 - 2^n 2^a X3^a Y^n 3^n4^{a}4^a; F = 2n = 6-2^n2^a X3^a X3^{a}4^{a}4^a) and Type 6 (M = 2n = 7 - 2^n 2^a Y3^a X^n 3^n 4^n 4^n ; F = 2n = 8 - 2^n 2^a X^n X^n 3^n 3^n 4^n 4^n). During the evolution of these karyotypes some of the parental chromosomes eliminated and some of them were retained.

Introgressive hybridization is more common in plants, and appears rarer in animals than plants at approximately 10% of species in major faunal groups (Mallet, 2007). To evolve this kind of cytoraces in nature it would have taken 1000s of years, whereas, here in the environs of laboratory it has taken only a decade. Therefore, lifespan study in these unique cytoraces is interesting as they are the hybridization products with introgressed genomes. The purpose of this study was to determine lifespan and its survivorship in all the members of *NAC* of *Drosophila*. Interracial differences, differences with their respective parents and the response to dietary restriction were studied. The result of this study will contribute to the knowledge of evolutionary theory of aging in *Drosophila*.

Materials and Methods

The following stocks were used in the present investigations:

a. Drosophila nasuta nasuta (N) (Coorg, India)
b. Drosophila nasuta albomicans (A) (Okinawa strain, Texas collection, USA, 3045.11)
c. Cytoraces 1 to 16 (Ramachandra and Ranganath, 1986, 1996)

Lifespan assessment

Stocks were maintained in half-pint bottles on standard molasses-agar-cornmeal medium supplemented by yeast at 22ºC. For the regular lifespan assessment, all the above mentioned stocks were maintained in five replicate bottles. Flies in the culture bottles were allowed to mate and lay eggs for around seven days and flies were removed. Then bottles with fertilized eggs were used to collect virgin flies after 20 days. Lifespan assessment was carried in three replicates using a modified protocol of Luckinbill and Clare (1985). For each of the replicate assessment, thirty unmated males and virgin females were collected and maintained separately in the vials with standard food medium supplemented with yeast (15mg per vial). Every alternate day, each male and female fly were transferred to fresh vial, mortality was recorded daily, likewise, a series of changes were made until all flies died.

Lifespan validation experiment

To understand the stability in lifespan of short-lived and long-lived cytoraces, we carried out four crosses (A, B, C, D). Each crosses experiment was carried with five pairs of unmated males and virgin females. Cross A - cytorace 3 males and cytorace 15 females; Cross B - cytorace 15 males and cytorace 3 females; Cross C - cytorace 2 males and cytorace 9 females; Cross D - cytorace 9 males and cytorace 2 females. Each pair was allowed to mate for seven days. Flies were then removed and vials of fertilized eggs were kept at 22ºC until the F1 generation began to emerge. Thirty unmated males and virgin females from each cross were collected separately and maintained in the vials with standard food medium supplemented with yeast (15mg per vial). Every alternate day each male and female fly were transferred to fresh vial, mortality was recorded daily, likewise, a series of changes were made until all flies died.

Dietary restriction (DR)

DR was made by the dilution of yeast the major food constituent in the food medium of Drosophila. In the standard diet, 15mg of yeast was provided in each media vial and it is been reduced to 2mg in DR. Concentration of yeast was reduced by employing the method of Mair *et al.* (2005) with slight modifications.

For this experiment, the assessment of lifespan remains same as standard diet (with 15 mg of yeast per vial) experiment except the concentration of yeast provided in each vial.

Statistical Analysis

Lifespan analyses were performed using SPSS Version 10.0. Data for lifespan assessment was subjected to One-Way ANOVA with races being treated as the fixed factor. Kaplan Meier analysis is used to compare the survival of two or more groups and log-rank test is used to compare the survival distribution; and the survival curves show time or age on X-axis and the portion of all individuals surviving on Y-axis. Kaplan Meier survival analysis and log-rank test was conducted by using MedCalc software (version 10.4.3; http://www.medcalc.be).

Survivorship (l_x) was also measured, which is a measure of the proportion of individuals which survive to the beginning of age category x, and it was estimated as $l_x = n_x / n_0$, where n_x is the number of individuals in the study population which survive to the beginning of age category x, and $n_0 = N$ (the total population size). (http://mathworld.wolfram.com/LifeExpectancy.html).

Results

Lifespan assessment in three replicates

Lifespan assessment in all the members of NAC of Drosophila revealed differences in the mean lifespan ranging from 46.87 days to 99.04 days in males and 52.45 and 119.48 days in females. One-Way ANOVA of lifespan among the unmated males (df=17, F=181.744, P < 0.001) as well as virgin females (df=17, F=207.308, P < 0.001) of all the members of NAC of Drosophila indicated significant differences. Virgin females showed significantly longer lifespan than the unmated males (df=17, F=129.794, P < 0.001) in all the members of NAC of Drosophila. Log-rank test indicated nonsignificant differences among three replicates for lifespan in all the members of NAC of Drosophila (Table 1). Among all the cytoraces of NAC of Drosophila, both unmated males and virgin females of cytoraces 3 and 15 lived shorter and cytoraces 2, 9, 11 and 16 lived longer than any other cytoraces.

Table 1: Summary of the log-rank test conducted for the lifespan in three replicates in all the members of *nasuta-albomicans* complex of *Drosophila*. Thirty unmated males and virgin females were assessed for their lifespan in each replicate.

Races	Log-rank test among three replicates (in males)		Log-rank test among replicates (in females)	
	χ^2	*P*-value	χ^2	*P*-value
D. n. nasuta	0.0038	0.99	3.3563	0.19
D. n. albomicans	4.1892	0.12	1.2878	0.52
Cytorace-1	0.4983	0.78	0.1312	0.94
Cytorace-2	4.9356	0.08	2.6734	0.26
Cytorace-3	0.1547	0.92	2.7813	0.25
Cytorace-4	0.0324	0.98	0.1367	0.93
Cytorace-5	0.7716	0.68	1.9336	0.38
Cytorace-6	3.3475	0.19	1.1845	0.55
Cytorace-7	2.2420	0.33	1.1943	0.55
Cytorace-8	2.6657	0.26	0.7729	0.68
Cytorace-9	1.7750	0.41	0.2012	0.90
Cytorace-10	0.6872	0.71	0.3968	0.82
Cytorace-11	3.3754	0.19	4.7481	0.09
Cytorace-12	3.5555	0.17	1.6341	0.44
Cytorace-13	0.5085	0.77	1.6344	0.44
Cytorace-14	0.5491	0.76	1.0113	0.60
Cytorace-15	0.4044	0.82	0.6657	0.72
Cytorace-16	3.1525	0.21	0.4548	0.80

Comparison of lifespan by One–Way ANOVA in all the cytoraces with their respective parents revealed the following (Table 2): Both males and females of cytoraces 1, 2, 4, 8, 9, 10, 16, and only males of cytoraces 6, 12, 13, 15 and females of cytoraces 5, 11, and 14 showed greater lifespan than their parents, hence these were considered as positively transgressive. Females of cytoraces 3, 6, 12, 13 and 15; males of cytorace 11; as well as, both males and females of cytorace 7 were negatively trangressive for lifespan than their parents. Males of cytoraces 3, 5, and 14 were parental-like, since they did not show any significant differences in lifespan.

Table 2: Mean values ± SE of lifespan assessed in all the members of *nasuta-albomicans* complex of *Drosophila* along with One-Way ANOVA. Positive or negative transgressive indicates significantly longer or shorter lifespan of the cytoraces than their parents respectively, and if the value do not differs significantly from the parents then referred as parental-like. This classification is according to Schwarzbach *et al.*, (2001).

Mean Longevity of					One-Way ANOVA		
Cytoraces			Parents of Cytoraces		*F*-value	*P*-value	Transgressiveness
C1	M	73.19 ± 1.14	NM	47.65 ± 1.06	269.56	0.001	*+ve transgressive*
	F	96.57 ± 1.50	AF	59.62 ± 0.38	573.86	0.001	*+ ve transgressive*
C2	M	90.72 ± 0.63	AM	50.52 ± 0.60	2170.25	0.001	*+ ve transgressive*
	F	114.91 ± 0.68	NF	54.64 ± 0.77	3419.51	0.001	*+ ve transgressive*
C3	M	46.87 ± 0.96	NM	47.65 ± 1.06	0.30	0.582	*parent like*
	F	52.45 ± 0.58	AF	59.62 ± 0.38	104.80	0.001	*- ve transgressive*
C4	M	67.45 ± 0.61	AM	50.52 ± 0.60	399.52	0.001	*+ ve transgressive*
	F	87.54 ± 0.97	NF	54.64 ± 0.77	698.58	0.001	*+ ve transgressive*
C5	M	74.80 ± 0.87	C1M	73.19 ± 1.14	1.27	0.262	*parent like*
	F	87.97 ± 0.66	AF	59.62 ± 0.38	1379.76	0.001	*+ ve transgressive*
C6	M	74.19 ± 0.75	C4M	67.45 ± 0.61	48.88	0.001	*+ ve transgressive*
	F	86.74 ± 0.80	C1F	96.57 ± 1.50	33.68	0.015	*- ve transgressive*
C7	M	62.24 ± 0.55	C1M	73.19 ± 1.14	74.83	0.001	*- ve transgressive*
	F	78.10 ± 0.81	C2F	114.91 ± 0.68	1209.85	0.001	*- ve transgressive*
C8	M	87.41 ± 0.60	C1M	73.19 ± 1.14	121.97	0.001	*+ ve transgressive*
	F	100.34 ± 0.79	C4F	87.54 ± 0.97	103.43	0.001	*+ ve transgressive*
C9	M	99.04 ± 0.61	C2M	90.72 ± 0.63	90.12	0.001	*+ ve transgressive*
	F	119.48 ± 0.97	NF	54.64 ± 0.77	2717.19	0.001	*+ ve transgressive*
C10	M	55.14 ± 0.57	C3M	46.87 ± 0.96	54.519	0.001	*+ ve transgressive*
	F	67.40 ± 0.85	NF	54.64 ± 0.77	122.30	0.001	*+ ve transgressive*
C11	M	88.72 ± 0.52	C2M	90.72 ± 0.63	5.97	0.016	*-ve transgressive*
	F	103.80 ± 0.76	AF	59.62 ± 0.38	2662.48	0.001	*+ ve transgressive*
C12	M	58.24 ± 0.38	AM	50.52 ± 0.60	119.74	0.001	*+ ve transgressive*
	F	69.62 ± 0.51	C1F	96.57 ± 1.50	291.74	0.001	*- ve transgressive*
C13	M	53.42 ± 0.70	AM	50.52 ± 0.60	9.91	0.002	*+ ve transgressive*
	F	60.75 ± 0.69	C2F	114.91 ± 0.68	3091.90	0.001	*- ve transgressive*
C14	M	68.97 ± 0.88	C4M	67.45 ± 0.61	1.99	0.159	*parent like*
	F	85.96 ± 0.74	C3F	52.45 ± 0.58	1266.71	0.001	*+ ve transgressive*
C15	M	49.52 ± 0.44	C3M	46.87 ± 0.96	6.26	0.013	*+ ve transgressive*
	F	52.95 ± 0.54	C4F	87.54 ± 0.97	964.08	0.001	*- ve transgressive*
C16	M	90.24 ± 0.59	NM	47.65 ± 1.06	1245.28	0.001	*+ ve transgressive*
	F	107.15 ± 0.61	C3F	52.45 ± 0.58	4189.87	0.001	*+ ve transgressive*

M = Male; F = Female

The survivorship of all the members of NAC of Drosophila was plotted against age in days in Figure 1a-b. The dotted lines indicate reduced survival, whereas, plain lines indicate the highest survival in the members of NAC of Drosophila. Females showed higher survival than males in all the members of NAC of Drosophila. In both males and females of D.n.nasuta, D.n.albomicans, cytoraces 3, 7, 10, 12, 13 and 15 survivability reduced as compared to cytoraces 1, 2, 4, 5, 6, 8, 9, 11, 14 and 16, in which it got extended.

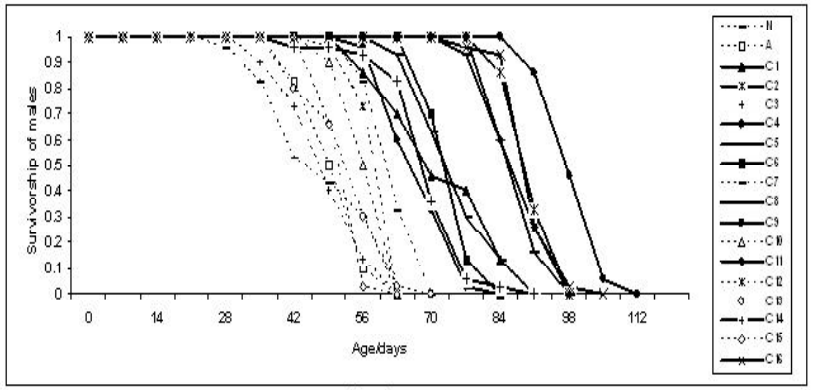

Fig. 1a

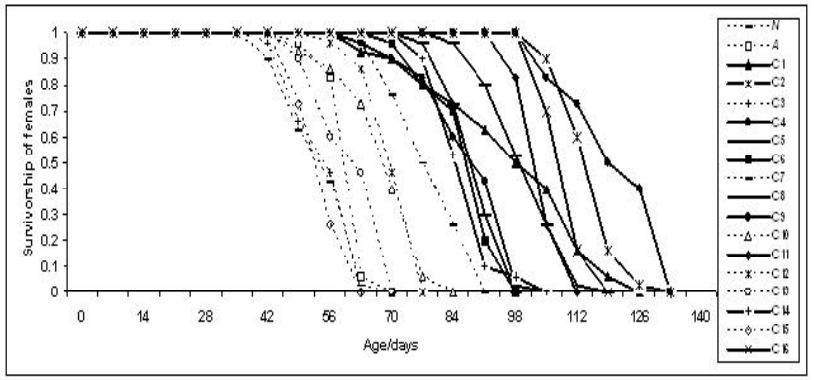

Fig. 1b

Figure 1a-b: Survivorship (lx) analysis of lifespan of males (a) and females (b) of all the members of *nasuta-albomicans* complex of *Drosophila*. Dotted lines (—) denote survival curve of cytoraces with reduced survival; plain lines denote the lifespan of cytoraces with higher survival.

Validation of short-lived and long-lived cytoraces

In order to further confirm the stability of lifespan in both short-lived and long-lived cytoraces, crosses were conducted between short-lived cytoraces (CROSS A: cytorace 3 male x cytorace 15 female; CROSS B: cytorace 15 male x cytorace 3 female) and between long-lived cytoraces (CROSS C: cytorace 2 male x cytorace 9 female; CROSS D: cytorace 9 male x cytorace 2 female). Lifespan of F1 offspring of these crosses were recorded and comparison was made between their respective parents by log-rank test (Table 3) which showed nonsignificant differences. Kaplan Meier survival curve was also plotted (Fig. 2a-d) to record the distribution of survival against age in days. In all the offspring of the four crosses and their parents there were nonsignificant differences in their survivorship.

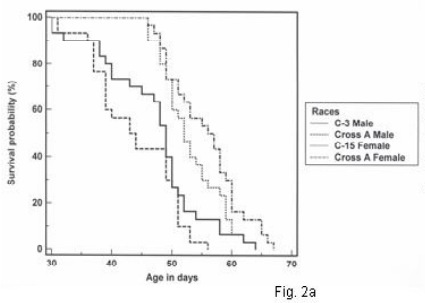

Fig. 2a

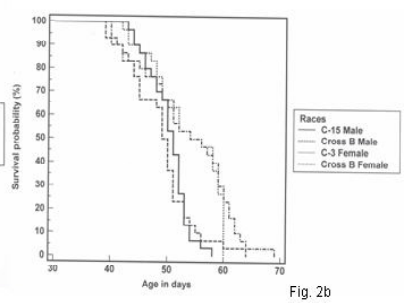

Fig. 2b

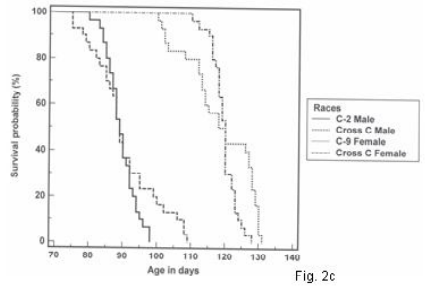

Fig. 2c

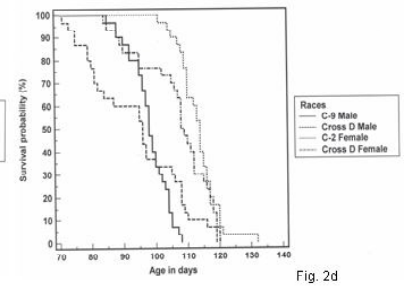

Fig. 2d

Figure 2a-d : Kaplan Meier survivorship curve of F1 offsprings of crosses with their respective parents: Figure 2a : Males and females of F1 offsprings of cross A with their male parent - cytorace 3 and female parent - cytorace 15; Figure 2b: Males and females of F1 offsprings of cross B with their male parent - cytorace 15 and female parent - cytorace 3; Figure 2c: Males and females of F1 offsprings of cross C with their male parent - cytorace 2 and female parent - cytorace 9; Figure 2d: Males and females of F1 offsprings of cross D with their male parent - cytorace 9 and female parent - cytorace 2.

Table 3: Comparative analysis of lifespan between offspring of crosses A, B, C and D and their respective parent using log-rank test. Thirty flies assessed separately for each cross.

Crosses	Parents	F1 offspring	Log-rank test	
			χ^2	*P*-value
Cross A	C-3M × C-15F	M	2.37	0.12
		F	3.60	0.06
Cross B	C-15M × C-3F	M	0.14	0.71
		F	1.05	0.30
Cross C	C-2M × C-9F	M	1.65	0.20
		F	3.35	0.07
Cross D	C-9M × C-2F	M	0.61	0.43
		F	3.08	0.08

One-way ANOVA among the F1 offspring of all the crosses (A, B, C and D) showed significant differences (?: df=3, MS- 21237.656, F-value- 220.643, P=0.001 and ?: df=3, MS-35125.044, F-value- 584.717, P= 0.001).

Influence of DR on short-lived and long-lived cytoraces

All the members of NAC of Drosophila were further subjected to DR in order to understand its effect on lifespan. The mean lifespan in response to DR extended significantly in both males and females of all the members of NAC of Drosophila except the long-lived cytoraces 2, 9, 11 and 16 (Table 4). The extension of lifespan in response to DR was maximum in D.n.nasuta, D.n.albomicans, cytorace 3 and cytorace 15, than any other cytoraces. In respect to this, comparisons of survivorship between standard diet and restricted diet were plotted for D.n.nasuta, D.n.albomicans and two short-lived and four long-lived cytoraces (Fig.3a-d). The rate of survivorship increased remarkably in DR in all short-lived cytoraces as compared to standard diet, whereas no such differences were noted in the survivorship in all the four long-lived races.

Table 4: One-Way ANOVA of lifespan between standard diet and dietary restriction in both males and females of the members of *nasuta-albomicans* complex of *Drosophila* (df =1 and *= P<0.001).

Races	One-Way ANOVA			
	Males		Females	
	Mean Square	*F*-value	Mean Square	*F*-value
D. n. nasuta	63700.41	417.84*	38253.75	319.83*
D. n. albomicans	54180.15	506.78*	29437.35	249.29*
Cytorace-1	14106.67	90.78*	6489.60	29.95*
Cytorace-2	380.02	2.69	8.067	0.11
Cytorace-3	130480.07	257.01*	61376.02	181.85*
Cytorace-4	59220.42	760.50*	17922.82	198.05*
Cytorace-5	19983.75	93.97*	5453.07	125.03*
Cytorace-6	22854.02	1000.53*	7843.27	174.73*
Cytorace-7	43040.82	1331.51*	5606.67	138.78*
Cytorace-8	2208.27	21.96*	224.27	2.34*
Cytorace-9	52.27	0.83	72.60	0.99
Cytorace-10	10962.02	117.50*	9151.35	50.51*
Cytorace-11	114.82	3.42	6.02	0.55
Cytorace-12	29703.75	463.96*	8616.02	281.81*
Cytorace-13	66733.35	472.08*	29659.27	163.90*
Cytorace-14	6489.60	134.15*	2829.07	62.42*
Cytorace-15	85957.35	325.27*	68141.40	1327.60*
Cytorace-16	132.02	4.39	22.82	1.34

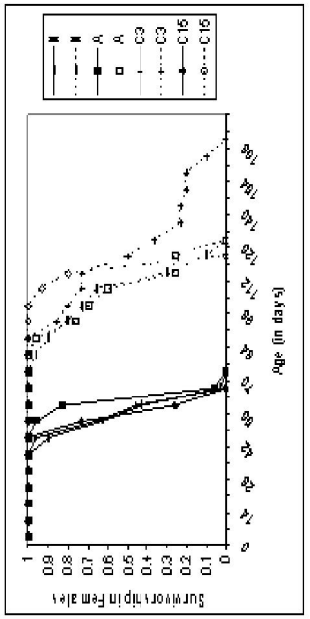

Fig. 3b

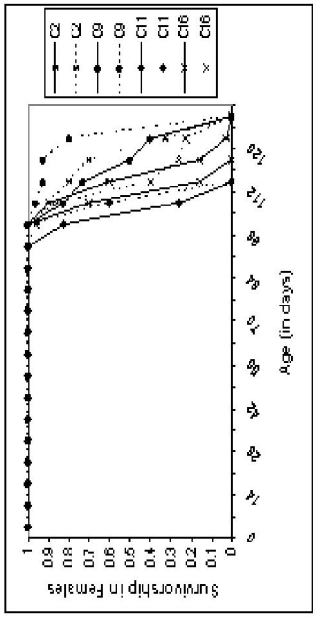

Fig. 3d

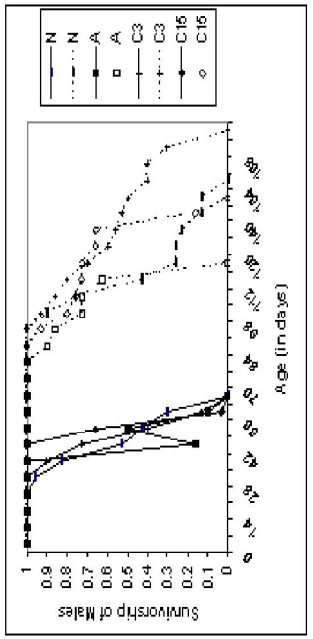

Fig. 3a

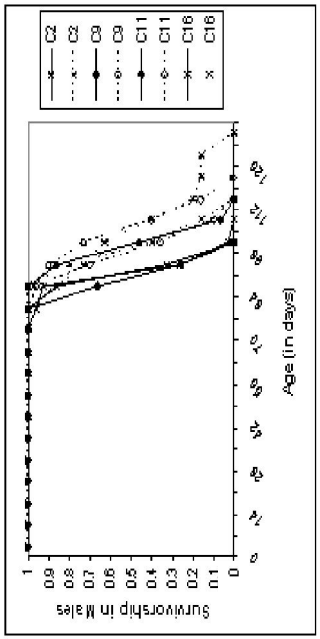

Fig. 3c

Figure 3a-d : Survivorship (lx) of standard lifespan and dietary restricted lifespan in males and females of D.n.nasuta, D.n.albomcians and two short-lived (Fig. 3a and 3b) and four long-lived cytoraces (Fig. 3c and 3d). Plain line denotes standard lifespan and dotted line (—) denotes dietary restricted lifespan.

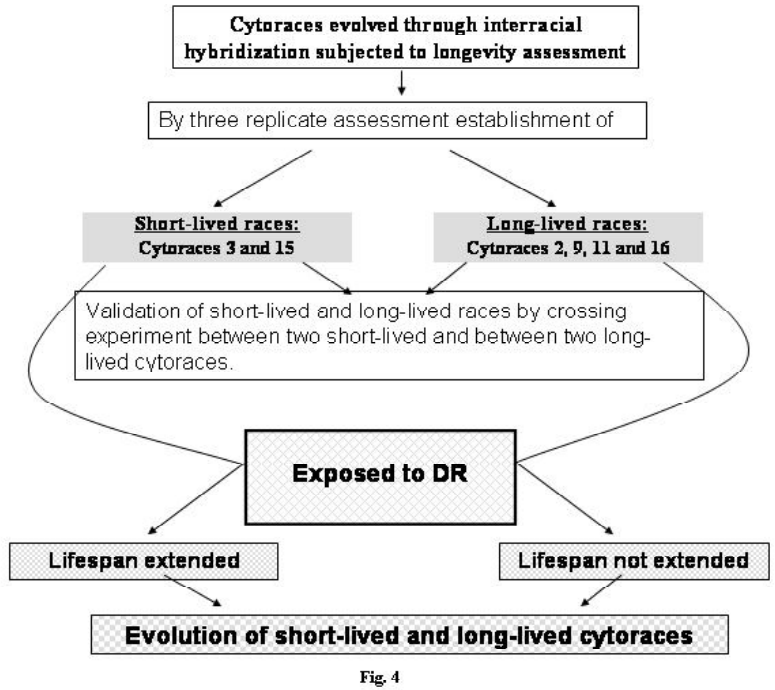

Fig. 4

Figure 4: Schematic representation of evolution of short-lived and long-lived cytoraces in Drosophila in the environs of laboratory.

Discussion

Evolutionary theories of aging and longevity are those theories that try to explain the remarkable differences in observed aging rates and longevity records across different biological species (Gavrilov and Gavrilova, 2002). The problem of the biological evolution of aging was initially studied in a purely theoretical, nonexperimental way. On the contrary, the evolutionary plasticity of aging and longevity is now an established experimental fact (Gavrilov and Gavrilova, 2002; Partridge and Gems, 2007). Determination of lifespan to assess the value of specific genetic alterations has important contribution (Rogina and Helfand, 2004). In the present study, the racial differences were observed with respect to lifespan in the members of NAC of Drosophila. Lifespan of cytoraces have been compared with their respective parents and categorized according to Schwarzbach et al., (2001) as positive or negative transgressive which indicates significantly greater or lesser

lifespan of hybrids than their parents, respectively, and if the value does not differ significantly from the parents, it is referred as parental-like. Such types of transgressiveness was recorded in our cytoraces, however, majority of the cytoraces are positively transgressive for lifespan than their parents.

Derivation of short-lived and long-lived cytoraces

Among all the cytoraces of NAC of Drosophila, cytoraces 3 and 15 showed shorter lifespan and cytoraces 2, 9, 11 and 16 showed longer lifespan than any other cytoraces. However, the remaining showed intermediate lifespan. These cytoraces have also shown differences in lifespan from their parents. In addition, these findings with the earlier assessment of Harini and Ramachandra (2003) have shown significant differences in the lifespan of cytoraces 1, 2, 9, 11 and 16. The pertinent question to be answered is : are the cytoaces stabilized for the longevity trait or still evolving? To address this question two additional lifespan assessments were carried out which revealed nonsignificant differences among cytoraces and derived two short-lived 3 and 15 as well as, four long-lived 2, 9, 11 and 16 cytoraces.

Another experiment was conducted to further validate the stability in the lifespan through crosses between two short-lived cytoraces i.e., cytoraces 3 and 15, as well as between two long-lived cytoraces 2 and 9. The reason behind this attempt was to know, whether the F1 offsprings of short-lived and long-lived races deviate from their parents? Interestingly, there is no heterosis, and no significant differences in the lifespan between F1 offspring of crosses and their respective parents; this strongly supported the stability of lifespan in cytoraces.

Influence of DR on lifespan

Dietary restriction is considered as a potent regimen that increases longevity in different organisms (Heilbronn and Ravussin, 2003; Kuobova and Guarente, 2003; Mair *et al.*, 2005; Heydari *et al.*, 2007). In the present study, the approach was to know whether DR has any influence on lifespan of cytorarces? Does DR enhance longevity in short-lived cytoraces? Does it disclose the secret behind living long? Can dietary restriction be considered as an important player in dictating the hidden secret of lifespan in our laboratory evolved hybrid races? We report racial divergence in the lifespan with response to DR. The

influence of DR on D.n.nasuta, D.n.albomicans, and two short-lived races cytoraces 3 and 15 is tremendous which has extended their lifespan significantly than the standard diet, whereas, unlike of other cytoraces, surprisingly, cytoraces 2, 9, 11 and 16 which have lived longer in the standard diet have not extended their lifespan further in response to DR. This indicates that cytoraces are unique introgressed products thereby exhibiting differential response to DR.

Since the short-lived and long-lived cytoraces have gathered different chromosomes from their parents it has an important input in determining lifespan. These cytoraces have not retained or eliminated all the chromosomes of D.n.nasuta or D.n.albomicans. When we compare the chromosome complements of cytoraces with their parents, the following observations can be made: 1) All these cytoraces are stabilized with heteromorphic second chromosomes indicating the balancing selection, by being retained with both the parental second chromosome; 2) All the short-lived and long-lived cytoraces possess D.n.albomicans dot chromosomes indicating the action of directional selection to retain only D.n.albomicans dot chromosomes; 3) Cytoraces 3, 15 and 16 exclusively retain third and X-chromosomes of D.n.nasuta, whereas, cytoraces 2, 9, 11 retained X3 and Y3 chromosomes of D.n.albomicans, here selection has favored both kinds of sex chromosomes indicating the mosaic selection. One of the possible explanations for this could be genomic stability in the cytoraces. During evolution, the favoured chromosomes have undergone unique kind of recombination and fixed the favorable new haplotype segments in short-lived and long-lived cytoraces from the parental species via hybridization and allowed the cytoraces to evolve differently with respect to the lifespan by being colonized with novel introgressed genomes (Fig. 4). Similarly, Baack and Rieseberg (2007) reported in plants the impact of hybridization on genomic stability; which can result in genomic changes including alterations to gene expression, chromosomal structure and genome size. Therefore, large magnitude of rapid genomic changes has caused differential lifespan and its response to DR. Hence, DR is considered to have played an important role in this study in substantiating the evolution of short-lived and long-lived cytoraces of NAC of Drosophila.

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References

Baack, E.J. & Rieseberg L.H. (2007). A genomic view of introgression and hybrid speciation. Curr Opin Genet Dev. 17(6): 513–518.

Chapman, T. & Partridge, L. (1996). Female fitness in Drosophila melanogaster: an interaction between the effect of nutrition and of encounter rate with males. Proc R Soc Lond B Biol Sci. 263: 755–759.

Charlesworth, B. (1993). Evolutionary mechanisms of senescence. Genetica. 91: 11–19

Chippindale, A., Leroi, A., Kim, S. & Rose, M. (1993). Phenotypic plasticity and selection in Drosophila life-history evolution. I. Nutrition and the cost of reproduction. J Evol Biol. 6:171–193.

David, J.R. & Fouillet, P. (1971). Quantitative underfeeding of Drosophila:Effect on adult longevity and fecundity. Exp. Gerontol. 6: 249-257.

Durbin, E.J. & Yoon, J.S. (1986). Longevity in the Drosophila virilis Species Group. I. The D. wnfcPhylad. OhioJ. Sci. 86(1): 14-17.

Durbin, E.J. & Yoon, J.S. (1987). Longevity in the Drosophila virilis Species Group. II.The D. Montana Phylad. OhioJ. Sci. 87(3): 90-92.

Gartner, L.P. (1973). Radiation-induced life span shortening in Drosophila. Gerontologia 19: 295-302.

Gavrilov, L.A. & Gavrilova, N.S. (2002). Evolutionary Theories of Aging and Longevity. The Scientific World. 2: 339-356

Harini, B.P. & Ramachandra, N.B. (2003). Evolutionary experimentation through hybridization under laboratory condition in Drosophila: Evidence for recombinational speciation. BMC Evolutionary Bio. 3: 1-19.

Heilbronn, L.K. & Ravussin, E. (2003). Calorie restriction and aging: review of the literature and implications for studies in humans. Am J Clin Nutr. 78:361–9.

Heydari, A.R., Unnikrishnan, A., Lucente, L.V. & Richardson, A. (2007). Caloric restriction and genomic stability. Nucleic Acids Research. 35: 7485-7496.

Kuobova, J. & Guarente, L. (2003). How does calorie restriction work? Gene Dev. 17: 313–321.

Luckinbill, L.S. & Clare, M.J. (1985). Selection for lifespan in Drosophila melanogaster. Heredity. 55: 9–18.

Mair, W., Piper, M.D.W. & Partridge, L. (2005). Calories do not explain extension of life span by dietary restriction in Drosophila. PLoS Biol. 3: e223.

Mallet, J. (2007). Hybrid speciation. Review. Nature. 446: 279-283.

Northrop, J.H. (1925). The influence of intensity of light on the rate of growth and duration of life of Drosophila. J. Gen. Physiol. 9: 81-86.

Paaby, A.B. & Schmidt, P.S. (2009). Dissecting the genetics of longevity in Drosophila melanogaster. Fly. 3: 1-10.

Parkes, T.L. Elia, A.J., Dickinson, et al., (1998). Extension of Drosophila lifespan by overexpression of human SOD1 in motor neurons. Nature Genetics 19:171–174.

Parsons, P.A. (1977). Genotype-temperature interaction for longevity in natural populations of Drosophila simulans. Exp. Geront. 12: 241-244.

Partridge, L. & Gems, D. (2006). Beyond the evolutionary theory of ageing, from functional genomics to evo-gero. Trends in Ecology and Evolution 21:334-340.

Partridge, L. & Gems, D. (2007). Benchmarks for ageing studies. Commentary. Nature. 450: 165-167.

Pearl, R. & Parker, S.L. (1922). Experimental studies on the duration of life. V. On the influence of certain environmental factors on the duration of life in Drosophila. Amer. Nat. 56: 385-398.

Pearl, R., Miner, J.R. & Parker, S.L. (1927). Experimental studies on the duration of life. XI. Density of population and life duration in Drosophila. Amer. Nat. 61: 289-318.

Ramachandra, N.B. & Ranganath, H.A. (1986). The chromosomes of two races: Drosophila nasuta nasuta and Drosophila albomicana:IV. Hybridization karyotype repatterning. Chromosoma 93:243-248.

Ramachandra, N.B. & Ranganath, H.A. (1990). The chromosomes of two Drosophila races: Drosophila nasuta nasuta and Drosophila nasuta albomicana: V. introgression and the evolution of new karyotypes. Z Zool Syst Evolut-forsh (Germany) 28: 62- 68.

Ramachandra, N.B. & Ranganath, H.A. (1996). Evolution of nasuta - albomicans complex of Drosophila. Curr. Sci. 71: 515-517.

Rogina, B., Reenan, R.A., Nilsen, S.P. & Helfand, S.L. (2000). Extended life-span conferred by cotransporter gene mutations in Drosophila. Science 290:147–150.

Rogina, B. & Helfand, S. (2004). Sir2 mediates longevity in the fly through a pathway related to calorie restriction. PNAS. 101:15998-16003.

Schwarzbach, A.E., Donovan, L.A. & Rieseberg, L.H. (2001). Transgressive character expression in a hybrid sunflower species. American. Journal. Of. Botany. 88(2):270-277.

Sohal, R.S. & Weindruch, R. (1996). Oxidative stress, caloric restriction, and aging. Science. 273:59-63.

Tanuja, M.T., Ramachandra, N.B. & Ranganath, H.A. (2003). Hybridization and introgression of the genomes of Drosophila nasuta and Drosophila albomicans Evolution of new karyotypes. Genome. 46:605-611.

Tatar, M., Bartke, A. & Antebi, A. (2003). The endocrine regulation of aging by insulin-like signals. Science 299:1346–1351.

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http://www.medcalc.be/

http://mathworld.wolfram.com/LifeExpectancy.html